Solubilization of Cell Wall Polysaccharides from Olive Fruits into Treatment Liquids during Spanish Green Olive Processing

Ana Jiménez, Coral Sánchez-Romero, Rafael Guillén, Juan Fernández-Bolaños, and Antonia Heredia*

Departamento de Biotecnología de Alimentos Instituto de la Grasa (CSIC), Apartado 1078, 41012 Sevilla, Spain

During Spanish green olive processing, several samples of fruits and liquids were taken. Cell walls of olive fruits were isolated and fractionated, and the liquids were analyzed by HPLC. During the lye treatment and wash, an exchange of arabinans from carbonate-soluble and 4 M KOH-soluble fractions to the water-soluble was observed, and a partial solubilization into the liquids was quantified (the average molecular weight of portions solubilized was 6 kDa). The main change in pectins was a movement of homo- and rhamnogalacturonans from water-soluble and carbonate-soluble fractions to the imidazole-soluble fraction during lye and wash due to high pH, but another minor one occurred: a partial solubilization of alkali-soluble and cellulose-linked pectins during lye treatment, wash, and fermentation. Around 3 mg/fruit of cellulose was also solubilized, mainly during lye and wash. Only a small part of this was recovered as oligo- and/or polysaccharides of molecular weight >1000 kDa. A very accurate balance was made during the whole process, keeping in mind the presence of fermentation.

Keywords: *Spanish green olives; polysaccharides; cell wall; solubility*

INTRODUCTION

Spanish green olive processing involves two different treatments: a first step at high pH value (2% sodium hydroxide) and a lactic fermentation. Each step leads to changes in the composition and structure of the olive cell wall that have been widely studied in previous works (Jiménez et al., 1995, 1996; Sánchez-Romero et al., 1998a,b). However, very little work has been done on the processing liquids. Existing studies on brine are focused on evaluating changes during fermentation or on quantifying the amount of fermentable sugars by different methods, such as the reducing-sugars method (Rodríguez de la Borbolla, 1981; Rodríguez de la Borbolla and Rejano, 1978), or more recently by HPLC directly from deionized brines (Garrido et al., 1993; Sánchez et al., 1995). The aim of this work has been to study both elements involved in the processing—olives and the different processing liquids—and to establish a complete relationship and balance between them at every step of the processing. In this way, a more detailed knowledge of how and when the modifications in olive cell wall take place during Spanish green olive processing has been obtained.

MATERIALS AND METHODS

Olive Processing. Olive fruits (*Olea europaea pomiformis* var. Manzanilla) were harvested in the province of Sevilla (Spain) at the mature green stage and were processed using the Spanish style method (Fernández-Díez, 1985). This involves treating the olives with a 2% sodium hydroxide solution (lye) for 5−6 h, using 3.5 L of lye/5 kg of fruit. The olives are then washed twice using 3.5 L of water/5 kg of fruit each time. The first wash is short (2 h), the second is longer (20 h). Subsequently, the fruits are placed in an 11% sodium chloride solution (brine) in the same volume/fruit ratio as before. The concentration of this solution decreases as the salt penetrates into the fruit, and the equilibrium concentration is established at ∼6%. Lactic fermentation takes place in the solution. At the beginning of brining, the pH of the medium is ∼10 because of the residual lye and decreases to pH 7−6 after 2 or 3 days. Acidification then continues more slowly, depending on the progress of fermentation. When pH 4 is reached and there are no fermentable sugars in the medium (4 or 5 months after brining), the fruits are considered processed. For this experiment three containers of ∼50 kg were used, filled at a ratio of olives/liquid of 30:21.

Liquid Sampling. The liquids studied were lye, second wash, brine after salt equilibrium (1 week after brining, pH of the medium 7.3), brine halfway through the fermentation process (3 months after brining, pH 5.1), and brine at the end of fermentation (5 months after brining, pH 4.3). All of the liquids were frozen after sampling and stored at −20 °C until analysis. Once melted, the liquids were processed according to two methods: dialysis and cation exchange—the first to study oligo- and polysaccharides and the second to quantify the total amount of sugars in the liquids.

(1) Dialysis. Each liquid was neutralized with acetic acid (lye and wash) or sodium hydroxide (midfermentation and end-of-fermentation brines), depending on its pH (brine at equilibrium had pH 7). Samples were dialyzed against deionized water for 2 days with several changes of water per day (dialysis tubing cutoff 1000). They were then freeze-dried and dissolved in 750 μL of deionized water for injection in HPLC and for determination of their glycosidic composition by GC (Jiménez et al., 1994), after hydrolysis with TFA (Ruiter and Burns, 1987).

(2) Cation Exchange. Cations were eliminated from the liquids using 1 mL of Dowex 50W-X12 (H+). Each liquid (1 mL) was passed through the exchanger, and the column was washed with 5 mL of deionized water. In the effluents, neutral sugars were quantified by GC and colorimetry according to the anthrone method (Dische, 1962) and uronic acids colori-

* Author to whom correspondence should be addressed (e-mail ahmoreno@sc.cica.es).

10.1021/jf971032h CCC: $15.00
Published on Web 09/19/1998

Table 1. Amount of Neutral Sugars in the Processing Liquids, after Dialysis, Expressed as Milligrams/1000 Fruit[a]

	lye		wash		brine equilibrium		1/2 ferm brine		end ferm brine	
rhamnose	25.55 ± 6.43	**9**	102.30 ± 12.48	**11**	22.54 ± 0.70	**10**	34.61 ± 5.09	**15**	26.18 ± 3.82	**15**
fucose	ND	**0**	ND	**0**	ND	**0**	ND	**0**	ND	**0**
arabinose	62.73 ± 7.63	**22**	81.38 ± 7.80	**9**	14.88 ± 0.44	**7**	31.61 ± 3.66	**13**	18.74 ± 1.93	**11**
xylose	6.17 ± 0.96	**2**	21.52 ± 0.87	**2**	5.39 ± 0.14	**3**	9.28 ± 0.99	**4**	6.39 ± 0.57	**4**
mannose	4.01 ± 0.10	**1**	27.30 ± 6.40	**3**	6.78 ± 0.10	**3**	10.49 ± 0.81	**4**	11.65 ± 0.16	**7**
galactose	9.61 ± 0.67	**3**	44.72 ± 3.32	**5**	11.40 ± 0.08	**5**	32.54 ± 0.96	**14**	29.36 ± 0.36	**17**
glucose	178.24 ± 18.30	**62**	670.08 ± 61.79	**71**	154.11 ± 2.01	**72**	119.20 ± 2.93	**50**	85.08 ± 0.83	**48**
total	286.31 ± 33.89		947.30 ± 92.65		215.10 ± 0.75		237.72 ± 14.45		177.40 ± 7.67	
total (Dowex)	*10456.65 ± 440.28*		*35882.82 ± 5503.50*		*18051.48 ± 1638.82*		*2763.98 ± 684.88*		*440.28 ± 48.92*	

[a] Bold data correspond to the percentage of each sugar on the amount of total sugar quantified. Italic data correspond to the amount of neutral sugars quantified after the liquids are passed through Dowex exchanger. The data are the average value of four determinations ± SD.

metrically according to the hydroxybiphenyl method (Blumenkrantz and Asboe-Hansen, 1973).

HPLC Analysis. A Hewlett-Packard system, series 1100, was used, fitted with two different Tosohaas columns (7.8 mm i.d. × 30 cm) in sequence: TSK gel GMPWXL (dextran MW < 50000 kDa) and TSK gel G3000PWXL (dextran MW < 60 kDa). The equipment was completed with a system of filter and precolumn and a differential refractometer (Waters, series R-404) as detector. The eluent buffer was 0.1 M imidazole−HCl, pH 7, at a flow of 0.5 mL/min. Under these conditions, the system was calibrated with dextrans of 250, 70, and 6 kDa (Fluka), cellobiose, and glucose. The injection volume was 20 μL, and the fractions collected were of 0.25 mL each. The studied samples were very dark, so an assay was made for determination of the elution profiles of neutral sugars to eliminate the interference caused in the detector by the brown color of the sample. Two or three peaks were recovered from each sample, dialyzed (cutoff 1000) against deionized water, freeze-dried, and dissolved in deionized water to quantify uronic acids by colorimetry and neutral sugars by GC.

Olive Sampling. Olives were sampled at the same stages of processing as were liquids: fresh fruit (FF), after lye treatment (AL), after wash (AW), at brine equilibrium (BE), halfway through the fermentation (HF), and processed fruit (PF). They were frozen and stored at −20 °C until analysis.

Cell Wall Isolation and Fractionation. Cell wall was isolated according to Selvendran's method (Selvendran and O'Neill, 1987), modified for olive pulp (Sánchez-Romero et al., 1998a). Cell wall was fractionated into various groups of polysaccharides depending on their solubility in a sequence of solvents: water, imidazole−HCl, sodium carbonate, and 1 and 4 M potassium hydroxide. The fractions analyzed were water-soluble (WSF), imidazole-soluble (ISF), carbonate-soluble (CSF), 1 M KOH-soluble (K1SF), 4 M KOH-soluble (K2SF), and α-cellulose (CEL). Each fraction was analyzed to quantify neutral sugars (GC) and uronic acids (colorimetry).

Determination of Free Sugars. Fresh pulp was treated twice with 80% ethanol. After filtration, both extracts were collected and ethanol was eliminated under vacuum. The aqueous solution was defatted with hexane and concentrated. In aliquots, neutral sugars were quantified by colorimetry and GC and uronic acids by colorimetry.

RESULTS AND DISCUSSION

This work starts with the general analysis of the liquids, continues with a study by HPLC to determine their molecular weight, and is completed with a study of the different cell wall fractions at various steps of the processing to correlate the polysaccharides found in the liquids with those lost from the cell wall. A balance of material is established, keeping in mind the restriction implied by the presence of fermentation.

Global Composition of Polysaccharides in Processing Liquids. GC analysis revealed important differences in polysaccharide composition (Table 1). Regarding total sugars, the wash step solubilized most sugars, probably because this was longer than the lye treatment, the cell wall structure had already been affected, and the pH of the medium during washing was still very alkaline.

The major sugar in all the of liquids was glucose. However, it is interesting to note the considerable amount of arabinose quantified in the lye (22% of total sugars), a percentage that was ∼10% in all other processing liquids. The compositions of wash and brine at equilibrium were very similar, although much more material was solubilized during the former. However, as fermentation progressed, the composition of the liquids changed considerably. In the second sample of brine, glucose decreased markedly (23%), whereas rhamnose, arabinose, and galactose were more abundant (the level of arabinose and galactose was more than twice that in the equilibrium brine).

A general diminution of sugars was observed at the end of the fermentation process with respect to half fermentation—only mannose did not change. Galactose decreased very slightly, but all of the other sugars decreased ∼30%. Arabinose was lost to a greater extent (60%).

The decrease in the amount of glucose during fermentation is normal, because this sugar is the raw material for the predominant microbial metabolism in this kind of lactic fermentation. However, as fermentation goes on, new polysaccharides appear in the brine. These polysaccharides, rich in rhamnose, arabinose, and galactose, could originate from two sources: olive pulp or microorganisms. If from the former, they are produced either during fermentation or during the lye/wash treatment but need >1 week to be released from the pulp to the medium. The acidic characteristics of the brine at the end of fermentation (pH ∼4 and 0.8−0.9% lactic acid) could be a cause of the final diminution of sugars. The smallest decrease shown by galactose could be the result of overlapping consumption and contribution in the medium. The largest decrease shown by arabinose is because this pentose and its polysaccharides (arabinans) are very sensitive to the acidic pH of fermentation (Stephen, 1983; Darvill et al., 1980).

From a comparison of the data of neutral sugars quantified after dialysis and after deionization by Dowex (Table 1), it is remarkable that most of the sugars in the liquids are of very low molecular weight (which are lost during dialysis). As fermentation progresses, both amounts become closer, because these short oligosaccharides are preferentially consumed by microorganisms. It is important to note that the main sugars

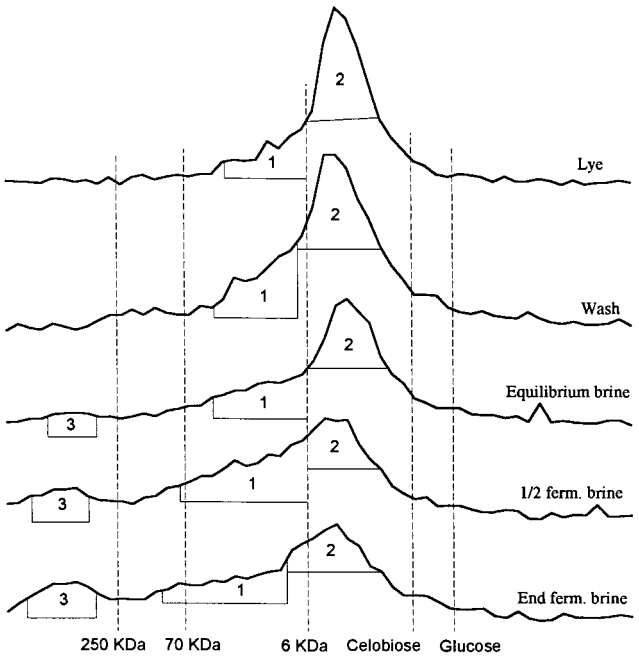

Figure 1. Profiles of 630 nm absorbance (neutral sugars) of the different processing liquids after HPLC analysis.

quantified in the liquids by GC using Dowex are glucose (75−80%) and mannose (20−21%), the same sugars and in nearly the same percentages as those comprising olive pulp free sugars (data not shown), and mannose arising from mannitol, a very abundant glycol in olive pulp (Guillén et al., 1992). Thus, the total amount of sugars in the processing liquids is mainly free sugars and, to a lesser extent, oligo- and polysaccharides released from the cell wall due to processing.

Analysis of Processing Liquids by HPLC. The profiles of absorbance at 630 nm (neutral sugars) of the samples analyzed are presented in Figure 1. From the lye and wash, two different fractions (fractions 1 and 2) were isolated, one above and the other below 6 kDa. The three brines contained, in addition to the fractions cited, another one with molecular weight >250 kDa (Fraction 3). The changes in sugar composition of the three fractions during processing are presented in Tables 2, 3, and 4, respectively.

The percentage of fraction 1 (Table 2) in the total sugars was the same in the early steps of processing (∼30%) but increased at the end of fermentation (reaching 39%). Its composition was very different in the samples analyzed. In the lye, arabinose was the major sugar quantified (37%), followed by glucose (28%). Rhamnose, uronic acids, and galactose represented 28% of the total sugars. In the wash, glucose was the most abundant, whereas arabinose decreased to 14% and did not change in the subsequent liquids. The sum of rhamnose, uronic acids, and galactose continued to increase throughout processing, due not to a decrease in the other main sugars (glucose and arabinose remained stable) but to an increase in their absolute amount, which changed from 28% in the lye to 49% in the brine at the end of fermentation; these polysaccharides are responsible for the increase of this fraction. The ratio Rha:Gal:UA also changed from 1.6:1:1.4 to 0.5:1:0.9, showing that galactose was the sugar that increased most.

The results for fraction 2 are presented in Table 3. This was the major fraction in all of the samples, but its percentage decreased from ∼70% in lye and wash to 50% at the end of fermentation. Glucose was always the major sugar, although its amount decreased during processing. The percentage of arabinose in the lye was again higher than in the other liquids, as in fraction 1. Glucose decreased 50% from equilibrium to the end of fermentation, but this diminution of ∼30 mg/1000 fruit

Table 2. Glycosidic Composition of Polysaccharides 70 kDa > MW > 6 kDa (Fraction 1) of the Different Processing Liquids after HPLC Analysis[a]

	lye		wash		equilibrium brine		1/2 ferm brine		end ferm brine	
mg/1000 fruit	61.22 ± 2.90 (31%)[b]		133.22 ± 6.50 (28%)		39.23 ± 3.34 (30%)		58.12 ± 6.29 (33%)		58.42 ± 3.90 (39%)	
rhamnose	6.54 ± 1.04	**11**	14.47 ± 0.23	**11**	5.51 ± 0.43	**14**	8.79 ± 1.91	**15**	5.63 ± 0.92	**10**
fucose	ND		ND		ND		ND		ND	
arabinose	22.83 ± 1.04	**37**	19.15 ± 0.39	**14**	5.15 ± 0.64	**13**	9.73 ± 0.87	**17**	8.57 ± 1.56	**15**
xylose	1.75 ± 0.10	**3**	6.91 ± 1.00	**5**	1.16 ± 0.41	**3**	1.36 ± 0.17	**2**	0.83 ± 0.21	**1**
mannose	1.92 ± 0.15	**3**	4.32 ± 0.44	**3**	1.61 ± 0.24	**4**	1.67 ± 0.04	**3**	3.50 ± 0.28	**6**
galactose	4.44 ± 0.66	**7**	12.24 ± 0.48	**9**	3.62 ± 0.01	**9**	10.73 ± 1.77	**18**	11.71 ± 0.44	**20**
glucose	17.39 ± 0.05	**28**	59.43 ± 1.09	**45**	15.14 ± 0.08	**39**	18.88 ± 1.07	**32**	17.22 ± 0.53	**29**
uronic acids	6.34 ± 2.55	**10**	16.70 ± 2.87	**12**	7.04 ± 1.54	**18**	6.67 ± 0.80	**11**	10.95 ± 1.02	**19**

[a] Bold data represent the percentage of each sugar on the amount of total sugar quantified. The data are the average value of four determinations ± SD. [b] The percentage in parentheses expresses the percent that the fraction represents on the total amount of sugars quantified in each liquid.

Table 3. Glycosidic Composition of Polysaccharides MW < 6 kDa (Fraction 2) of the Different Processing Liquids after HPLC Analysis[a]

	lye		wash		equilibrium brine		1/2 ferm brine		end ferm brine	
mg/1000 fruit	139.22 ± 5.47 (69%)[b]		341.49 ± 6.71 (72%)		87.77 ± 4.31 (66%)		85.48 ± 5.88 (49%)		75.44 ± 0.32 (50%)	
rhamnose	10.98 ± 0.81	**8**	20.28 ± 0.24	**6**	5.34 ± 1.17	**6**	9.44 ± 1.39	**11**	9.89 ± 0.32	**13**
fucose	ND		ND		ND		ND		ND	
arabinose	18.05 ± 1.75	**13**	13.99 ± 0.42	**4**	3.47 ± 0.41	**4**	7.64 ± 0.03	**9**	6.52 ± 0.24	**9**
xylose	2.85 ± 1.14	**2**	8.78 ± 0.39	**3**	2.28 ± 0.15	**3**	4.56 ± 0.01	**5**	3.31 ± 0.00	**4**
mannose	0.62 ± 0.02	**1**	7.57 ± 0.16	**2**	2.29 ± 0.26	**3**	3.85 ± 0.59	**4**	3.68 ± 0.15	**5**
galactose	2.46 ± 0.21	**2**	8.25 ± 0.31	**2**	2.52 ± 0.24	**3**	6.91 ± 1.50	**8**	6.95 ± 0.18	**9**
glucose	102.96 ± 0.71	**74**	266.13 ± 0.4	**78**	65.53 ± 5.32	**75**	42.37 ± 0.13	**50**	33.21 ± 0.29	**44**
uronic acids	1.29 ± 3.49	**1**	16.51 ± 4.79	**5**	6.35 ± 0.92	**7**	10.71 ± 2.25	**12**	11.88 ± 0.75	**16**

[a] Bold data represent the percentage of each sugar on the amount of total sugar quantified. The data are the average value of four determinations ± SD. [b] The percentage in parentheses expresses the percent that the fraction represents on the total amount of sugars quantified in each liquid.

Table 4. Glycosidic Composition of Polysaccharides MW > 250 kDa (Fraction 3) of the Different Processing Liquids after HPLC Analysis[a]

	equilibrium brine		1/2 ferm brine		end ferm brine	
mg/1000 fruit	4.81 ± 1.82 (4%)[b]		30.58 ± 4.97 (17%)		17.41 ± 0.81 (12%)	
rhamnose	ND		0.55 ± 0.02	**2**	1.42 ± 0.15	**8**
fucose	0.25 ± 0.01	**5**	0.41 ± 0.11	**1**	ND	
arabinose	0.11 ± 0.02	**2**	1.25 ± 0.39	**4**	0.51 ± 0.05	**3**
xylose	0.39 ± 0.09	**8**	1.36 ± 0.16	**4**	0.22 ± 0.02	**1**
mannose	0.56 ± 0.19	**12**	0.83 ± 0.02	**3**	0.51 ± 0.1	**3**
galactose	0.29 ± 0.03	**6**	3.02 ± 0.20	**10**	1.92 ± 0.00	**11**
glucose	3.08 ± 0.02	**64**	19.68 ± 1.00	**64**	9.40 ± 0.30	**54**
uronic acids	0.12 ± 1.45	**2**	3.49 ± 3.10	**11**	3.44 ± 0.69	**20**

[a] Bold data represent the percentage of each sugar on the amount of total sugar quantified. The data are the average value of four determinations ± SD. [b] The percentage in parentheses expresses the percent that the fraction represents on the total amount of sugars quantified in each liquid.

was greater than in the total fraction (12 mg/1000 fruit) because of the increase of other sugars, mainly uronic acids, rhamnose, galactose, and arabinose. The changes in the ratio Rha:Gal:UA were the same as in fraction 1, but the increase in galactose was smaller. In addition to glucose from free sugars, the glucose-rich polysaccharides present in this fraction are also a source of bacteria-consumable glucose.

Fraction 3 (Table 4) comprised mainly glucose, although in the equilibrium brine there was 12% mannose, with the other sugars in percentages <10%. The same behavior as in the other fractions was observed during fermentation: a decrease in glucose and an increase of rhamnose, galactose, and uronic acids. However, in this case, the maximum was obtained when fermentation was still active, and all sugars diminished at the end of fermentation.

Changes in Cell Wall Fractions during Processing. The composition of the different fractions studied is presented in Figures 2 (neutral sugars) and 3 (uronic acids). The effects of lye and wash will be discussed together as a single step, the same as for fermentation. In determinate cases, when the effects of lye and wash were different or when the behavior of samples halfway and at the end of fermentation showed differences, that specific step will be discussed separately.

During lye and wash, many changes took place. The increase in neutral sugars in WSF, and their diminution in CSF and K2SF, could be explained as a movement of arabinans. The loss of these polysaccharides from carbonate and highly alkaline fractions was quantified as 1.1 mg/fruit, and the increase in water was 0.84 mg/fruit. These data imply a solubilization of ~0.2 mg arabinans/fruit into lye and wash (Sánchez-Romero et al., 1998a). Cellulose underwent a marked decrease, but no increase of glucose was detected in the other fractions.

In the case of uronic acids, there was a clear exchange between fractions in these processing steps: UA decreased in WSF and CSF and increased in ISF. As a result of high pH, homogalacturonans in WSF were deesterified, and rhamnogalacturonans in CSF were released because of the breakdown of their ester linkages to the cell wall structure, both modified polysaccharides being soluble in imidazole (Jiménez et al., 1995, 1996; Sánchez-Romero et al., 1998a). During lye treatment, there was also an increase of UA in K1SF, pectic fractions of low molecular weight arising from the diminution of pectins in K2SF and CEL. K1SF underwent the opposite change during wash: UA decreased and was not recovered in other fractions. Probably, those fractions of rhamnogalacturonans released from

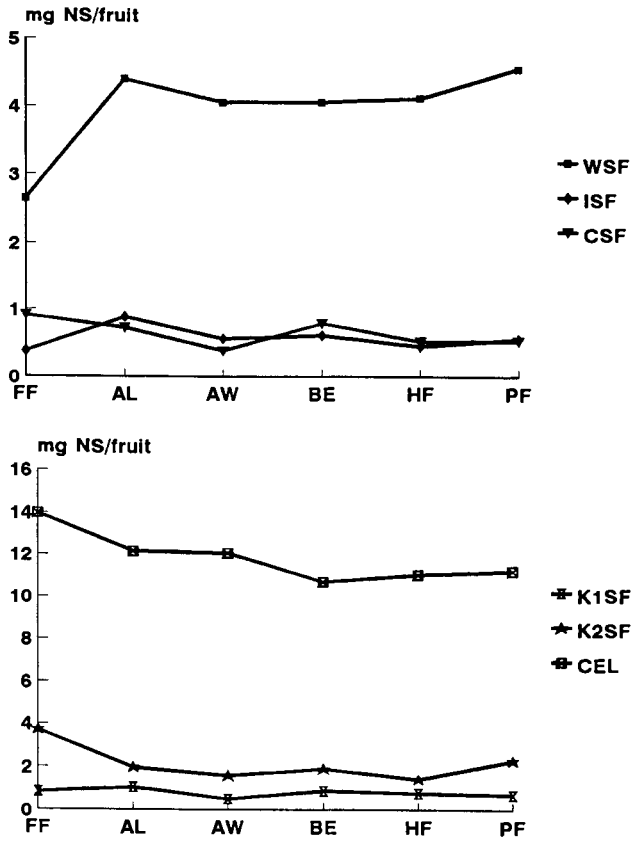

Figure 2. Evolution of neutral sugar amounts in cell wall fractions during olive processing.

K2SF and CEL during lye treatment are more modified as a result of such a long period at high pH, and these new and shorter fractions are lost into the processing liquids. The wash had the same effect on ISF—NS and UA decreased, and the fractions of arabinans and rhamnogalacturonans probably passed into the liquids.

During fermentation, due to low pH and enzymatic activity, the polysaccharides underwent more modifications. Cellulose lost uronic acids, which increased in WSF, and CSF lost neutral sugars, which were recovered in WSF. The increase of both components, neutral sugars and uronic acids, in WSF indicates that fractions of arabinans and rhamnogalacturonans were being recovered in that fraction from cellulose and CSF. Portions of those same polysaccharides but of lower molecular weight than those recovered in WSF could be solubilized into the brine from the same fractions.

It could be concluded that during lye and wash there are exchanges of arabinans and homo- and rhamnoga-

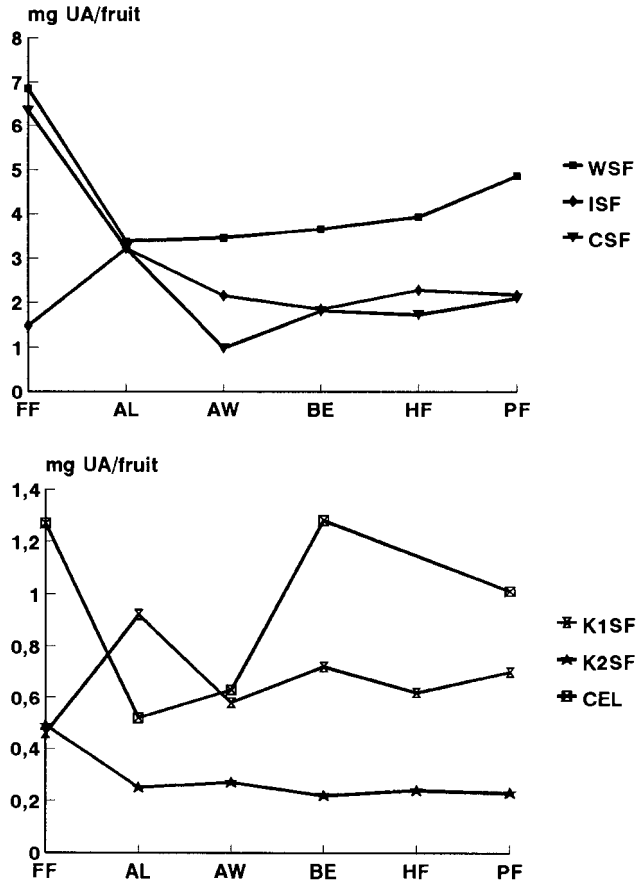

Figure 3. Evolution of uronic acid amounts in cell wall fractions during olive processing.

lacturonans between fractions and a minor solubilization into the processing liquids. There is also a considerable loss of cellulose. During fermentation, rhamnogalacturonans are the most affected, being released from fractions strongly linked to the cell wall structure into more soluble fractions and perhaps into the brine.

Overall Balance of Sugars. The data for neutral sugars and uronic acids are presented separately in Table 5. In the total amount of sugars, there are very good recoveries in neutral sugars and uronic acids. In neutral sugars, from the beginning of the fermentation process the total amount decreased because of bacterial metabolism. It is interesting that although the amount of sugars from the cell wall diminished to ~2 mg/fruit, the highest contribution to the sugars of the processing liquids was from free sugars, which decreased most during processing.

In uronic acids, there were no significant differences in the total amount quantified in each step of the processing, this always being ~11 mg/fruit (free sugars plus processing liquids plus cell wall). Thus, this component is not consumed or altered by microorganisms or medium conditions.

CONCLUSIONS

Previous works on this topic (Jiménez et al., 1995, 1996; Sánchez-Romero et al., 1998a,b) studied global changes between unprocessed and processed fruits. In pectins, the greatest variations were diminution of homo- and rhamnogalacturonans in the water- and carbonate-soluble fractions and increase in the imidazole-soluble one. These exchanges between fractions occurred without a decrease in molecular weight, by deesterification (from WSF to ISF) and by breakdown of ester linkages (from CSF to ISF) (Jiménez et al., 1996; Sánchez-Romero et al., 1998a). As can be concluded from this work, these changes take place mainly during lye treatment and wash (Figure 3), but they have no effect on the composition of the processing liquids. There were other minor changes in these polysaccharides that imply a degradation of the pectic backbone: diminution of pectins in K2SF and CEL and increase in K1SF. This increase was in polysaccharides of ~70−40 kDa (Sánchez-Romero et al., 1998a). The exchange of pectins and decrease in molecular weight took place during lye treatment in K2SF and also during fermentation in CEL (Figure 3). These results can be related to the composition of the liquid. Rhamnose, galactose and uronic acids are important sugars in the polysaccharides quantified in the processing liquids, accounting for between 11 and 49% of the total sugars, depending on the liquid and the fraction (Tables 2 and 3). Their molecular weights are <70 kDa (Figure 1), and the amounts of the three sugars were very similar in both fractions (above and below 6 kDa). Moreover, uronic acid decreases in K1SF during washing (Figure 3) and does not increase in other fractions. Thus, although the main change in pectins during Spanish style processing is the exchange between WSF, ISF, and CSF, from these results it can be concluded that another minor change takes place: a decrease in the molecular weight of alkali-soluble pectins and of pectins linked to cellulose, the shortest fractions of which could be solubilized into the processing liquids.

Table 5. Overall Balance of Sugars during Olive Processing (Milligrams/Fruit)[a]

	FF	AK	AW	BE	HF	PF
neutral sugars						
free sugars	64.91 ± 3.98	54.31 ± 3.80	29.95 ± 2.10	18.37 ± 0.73	1.63 ± 0.18	
processing liquids		10.46 ± 0.44	35.88 ± 5.50	18.05 ± 1.64	2.76 ± 0.68	0.44 ± 0.04
cell wall	22.00 ± 3.16	21.26 ± 1.81	19.19 ± 1.32	18.89 ± 2.32	18.78 ± 1.60	19.81 ± 0.95
total	86.91 ± 7.05 a	86.03 ± 6.05 a	85.02 ± 8.92 a	55.31 ± 4.69 b	23.17 ± 2.46 c	21.88 ± 1.17 c
uronic acids						
free sugars	0.68 ± 0.02	1.02 ± 0.04	0.35 ± 0.01	0.43 ± 0.15	0.61 ± 0.14	
processing liquids		0.17 ± 0.007	0.39 ± 0.07	0.39 ± 0.09	0.37 ± 0.003	0.32 ± 0.001
cell wall	11.83 ± 0.63	9.47 ± 0.34	9.80 ± 0.39	11.08 ± 0.38	9.81 ± 0.45	11.03 ± 0.84
total	12.51 ± 0.65 a	10.66 ± 0.39 a	10.45 ± 0.47 a	11.90 ± 0.62 a	10.79 ± 0.59 a	11.35 ± 0.84 a

[a] Data with the same letters indicate no significant differences. The amount of sugars in the processing liquids was quantified after passing through Dowex exchanger. The data are the average value of four determinations ± SD.

Arabinans are other polysaccharides affected by processing. As reported in a previous work (Sánchez-Romero et al., 1998a), there is an increase of 0.84 mg/fruit of arabinans in WSF and a total decrease of 1.1 mg/fruit in CSF and K2SF. Thus, apart from the arabinans recovered in the WSF, there must be a solubilization into the processing liquids of ~0.2 mg/fruit. From the data presented in this work, it is clear that the exchange takes place mainly during lye treatment and wash (Figure 2), as shown by the analysis of the processing liquids. In lye and wash, 0.14 mg/fruit was recovered: 0.06 mg/fruit in the lye and 0.08 mg/fruit in the wash (Table 1). In ~57% of these fractions of arabinans, the molecular weight is between 70 and 6 kDa, and in the rest is <6 kDa, in both lye and wash (Tables 2 and 3). During fermentation, arabinose is also present, but the balance in this case is more difficult to establish because the pentose and its polymers are very sensitive to the acidic pH of the medium. However, the second sample of brine contained 0.03 mg/fruit of arabinose, a value that could close the overall balance of arabinans, the fractions above and below 6 kDa being in the same ratio in the three brines as in the lye and wash.

As mentioned above, glucose is the major sugar in all of the liquids, analyzed both after Dowex and after dialysis, because it is the most abundant free sugar in olive fruit (~80%), 52 mg/fruit (data not shown). However, the presence of a minor amount of glucose-rich polysaccharides in the liquids after dialysis shows that this glucose cannot arise from free sugars but from cellulose. The decrease in cellulose quantified in a previous work (Sánchez-Romero et al., 1998b) takes place mainly during lye and wash treatments (1.91 mg/fruit) and during fermentation (0.86 mg/fruit), accounting for a total amount of 2.78 mg/fruit. Only part of this solubilized cellulose is recovered in the liquids as polymeric material not lost during dialysis (Table 1): 44% during lye and wash and 18% at the beginning of fermentation. A balance of glucose during processing cannot be established, because microorganism metabolism is able to consume glucose even from fractions of molecular weight <6 kDa (Table 3). However, during lye and wash, a very approximate balance of glucose could be made. The glucose losses are from the free sugar fraction (27 mg/fruit) and from the cellulose fraction (2 mg/fruit), and glucose quantified (after Dowex exchanger) in the liquids was 34 mg/fruit. The difference between these amounts could lie within the statistical variations of the methodology applied.

It could be concluded that the total balance of material, within such a complex process as the Spanish green olive method, has been established. The polysaccharides that are mainly affected have been identified, and the moment of processing when their modification takes place has been established. Spanish green olive processing can be considered a closed system in which some polysaccharides change their solubility characteristics, leading to exchanges between cell wall fractions or their solubilization into the liquids, where they are consumed or not by lactic bacteria.

ABBREVIATIONS USED

GC, gas chromatography; FF, fresh fruit; AL, fruits after lye treatment; AW, fruits after wash; BE, fruits at brine equilibrium; HF, fruits halfway through the fermentation; PF, processed fruit; WSF, water-soluble fraction; ISF, imidazole-soluble fraction; CSF, carbonate-soluble fraction; K1SF, 1 M KOH-soluble fraction; K2SF, 4 M KOH-soluble fraction; CEL, α-cellulose residue; UA, uronic acids; NS, neutral sugars.

LITERATURE CITED

Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem*. **1973**, *54*, 484−489.

Darvill, A.; McNeil, M.; Albersheim, P.; Delmer, D. P. The primary cell walls of flowering plants. In *The Biochemistry of Plants: A Comprehensive Treatise*; Tolbert, N. E., Ed.; Academic Press: New York, 1980; Vol. 1, pp 91−162.

Fernández-Díez, M. J. *Biotecnología de la Aceituna de Mesa*; Publicaciones CSIC: Sevilla-Madrid, 1985; pp 55−123.

Garrido, A.; García, P.; Montaño, A.; Brenes, M.; Durán, M. C. Biochemical changes during the preservation stage of ripe olive processing. *Nahrung* **1993**, *37*, 583−591.

Guillén, R.; Heredia, A.; Felizón, B.; Jiménez, A.; Montaño, A.; Fernández-Bolaños, J. Fibre fraction carbohydrates in *Olea europaea* (Gordal and Manzanilla var.). *Food Chem*. **1992**, *44*, 173−178.

Jiménez, A.; Guillén, R.; Fernández-Bolaños, J.; Heredia, A. Cell wall composition of olive fruit. *J. Food Sci*. **1994**, *59*, 1192−1196, 1201.

Jiménez, A.; Guillén, R.; Sánchez, C.; Fernández-Bolaños, J.; Heredia, A. Changes in texture and cell wall polysaccharides of olive fruit during "Spanish Green Olive" processing. *J. Agric. Food Chem*. **1995**, *43*, 2240−2246.

Jiménez, A.; Guillén, R.; Sánchez, C.; Fernández-Bolaños, J.; Heredia, A. Molecular weight and ionic characteristics of olive cell wall polysaccharides during processing. *J. Agric. Food Chem*. **1996**, *44*, 913−918.

Rodríguez de la Borbolla, J. M. On the preparation of Sevillan style olives. The lye treatment. *Grasas Aceites* **1981**, *32*, 181−189.

Rodríguez de la Borbolla, J. M.; Rejano, L. On the preparation of Sevillan style olives. The washing of fruits treated with lye. *Grasas Aceites* **1978**, *29*, 281−291.

Ruiter, J. M.; Burns, J. C. Characterization of trifluoroacetic acid hydrolyzed of subtropical forage grass cell walls. *J. Agric. Food Chem*. **1987**, *35*, 308−316.

Sánchez, A.; García, P.; Rejano, L.; Brenes, M.; Garrido, A. The effects of acidification and temperature during washing of Spanish-style green olives on the fermentation process. *J. Sci. Food Agric*. **1995**, *68*, 197−202.

Sánchez-Romero, C.; Guillén, R.; Heredia, A.; Jiménez, A.; Fernández-Bolaños, J. Degradation of pectic polysaccharides in pickled green olives. *J. Food Prot*. **1998a**, 78−86.

Sánchez-Romero, C.; Guillén, R.; Heredia, A.; Jiménez, A.; Fernández-Bolaños, J. Degradation of hemicellulosic and cellulosic polysaccharides in pickled green olives. *J. Food Prot*. **1998b**, 87−93.

Selvendran, R. R.; O'Neill, M. A. Isolation and analysis of cell walls from plant material. *Methods Biochem. Anal*. **1987**, *32*, 25−153.

Stephen, A. M. Other plant polysaccharides. In: *The polysaccharides*. Aspinall, G. O., Ed.; Academic Press: New York, 1983, Vol. 2, pp 97−193.

Received for review December 3, 1997. Revised manuscript received April 30, 1998. Accepted July 22, 1998. This work was supported by CICYT (ALI94-0980-C02-02).

JF971032H